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GRANT NUMBER: DAMD17-94-J-4294

TITLE: University of Kansas Cancer Center Breast Tissue and Serum Repository Core Facility

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REPORT DATE: October 1995

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1995	Annual 23 Sep 94 - 22 Sep 95

4. TITLE AND SUBTITLE
University of Kansas Cancer Center Breast Tissue and Serum Repository Core Facility

5. FUNDING NUMBERS
DAMD17-94-J-4294

6. AUTHOR(S)
Jonathan Li, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Kansas Medical Center
Kansas City, Kansas 66160

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The newly established Division of Etiology and Prevention of Hormonal Cancers, University of Kansas Cancer Center (KUCC), has developed in the first year of U.S. Army Medical Research and Development support a Breast Tissue and Serum Repository (BTSR) Core Facility to facilitate and foster breast cancer-related research at KUCC and other research institutions in the Southern Plains States. To date, the BTSR has collected multiple malignant breast cancer specimens and corresponding serum and lymphocyte specimens from the same patients. In regard to normal breast specimens collected for BTSR via breast reduction, thus far only one multiple specimen has been obtained. For each patient specimen, whether serum or tissue, a personal health history form has been completed. In addition, physician records of each patient are available if the information contained therein is needed by investigators. Patient confidentiality is strictly maintained, and patients' identities are not available to users of the BTSR Core Facility. A committee has been formed comprising both clinical and basic science faculty to review proposals for basic science and clinical studies.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast cancer, Breast Tissue and Serum Repository Core Facility	15
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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★U.S.GPO:1991-0-299-935

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PI - Signature

10/27/95

Date

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Introduction

The cause(s) of breast cancer and the means to predict who will develop it are currently not well understood. Understanding of either or both is an essential step to successful prevention of this prevalent disease in the future. Similarly, there is a paucity of knowledge related to early detection of breast cancer, because screening procedures, while improving, do not allow detection of breast cancer at the earliest and most curable stages. The development of the BTSR Core Facility at the KUCC-KUMC is an important step to address these issues at this institution.

Development of the BTSR has been and will continue to be highly relevant to expansion and augmentation of breast cancer research, including clinically-related and basic, at the University of Kansas Cancer Center (KUCC) and Medical Center (KUMC). The BTSR's purpose is to facilitate investigator-initiated research to perform retrospective epidemiologic studies on preserved material; to perform correlation studies on the incidence of possible premalignant and malignant breast lesions with genetic and variable biomarkers (e.g., receptors, hormones, cellular proteins, protooncogenes, and tumor suppressor genes, etc.); and to assess the presence of potential carcinogens.

A focus of the newly created Division of Etiology and Prevention of Hormonal Cancers (DEPHC) is to assist, complement, and expand existing, ongoing programs and to develop new programs in molecular biology and molecular cytogenetics in breast cancer research at KUCC. A central emphasis of this Division is that hormones, particularly estrogens and progestin, play a critical role in breast tumor causation, progression, and dependency. Hormonal involvement in breast cancer etiology at the cellular and molecular level is not well understood and requires elucidation.

Body

I. Background

The KUCC-BTSR was funded beginning September 23, 1994. Allocation of designated KUCC-BTSR space in the Pathology Department was delayed due to the unanticipated retirement of Dr. Reals, its chairman. The new chairman of Pathology, Dr. Damjanov, was unable to allocate the space designated in the proposal because of reorganizational problems within the department. Dr. Daniel Hollander, Dean of the Medical School, and Dr. Roger Lambson, Vice-Chancellor for Administration, solved this problem by allocating space in the new Lied Biomedical Research Facility. However, construction delays on that building prevented any of the DEPHC research staff from moving in until the end of January 1995. During this interim period, the successful recruitment of Dr. Walter Imagawa as Associate Director for the KUCC-BTSR was accomplished on October 2, 1994. Dr. Imagawa, an established breast cancer researcher from the Cancer Research Laboratory, University of California-- Berkeley, is involved in the day-to-day operation of the Core Facility. In addition, major and minor equipment and supplies for use in the KUCC-BTSR were ordered and received during this period. On May 8, 1995, Ms. Leslie Hudson was recruited in the Biologist II position, and on August 7, 1995, Ms. Rhonda Doolittle was recruited to handle the expanding secretarial work associated with the KUCC-BTSR Core Facility.

II. Experimental Methods

TISSUE SAMPLES

Tissue samples for the KUCC-BTSR are acquired from patients who have breast biopsies, lumpectomies, and/or mastectomies and also from women who have breast reduction surgery. Ms. Leslie Hudson, the BTSR biologist, acquires the daily surgical schedule for all breast surgeries and is present in the Surgical Pathology Laboratory during the processing of the breast specimens. These are handled in a timely fashion in order to preserve the tissues appropriately. The breast tissue, normal, abnormal, and neoplastic, is placed on a frozen cutting board provided by the BTSR. The breast tissue specimens are delivered to the Surgical Pathology Laboratory within 10 min. A certified pathologist immediately evaluates the tumor, and a frozen section is prepared for diagnosis. The pathologist then cuts tumor/normal tissue specimens for the repository biologist if sufficient sample is available.

If there is sufficient breast tissue sample, one portion is allocated for frozen sections. Tissue samples destined for frozen section are covered with tissue-embedding medium in a cryomold, then placed in an airtight polypropylene container, labeled with a proper bar-code label (specimen-specific identification number--please see below), and immediately snap-frozen in an N_2 container before storage in the BTSR freezer. The remaining tissue sample is similarly labeled and snap-frozen in a polypropylene container.

Each specimen is assigned a unique six-digit specimen-specific identification number. The six-digit number is assigned sequentially, with biopsy tissue, healthy adjacent tissue, and serum for a particular patient assigned the same number. All breast tissue aliquots derived from the same tissue are assigned the same six-digit number. This six-digit specimen-specific identification number is shown on the bar code with which the biologist labels each container and slide.

A Surgical Pathology requisition form is computer generated by the Surgery Department and accompanies the tumor specimen when it is delivered to the Pathology Laboratory. Information included on this form consists of hospital patient identification number, surgeon's name, patient's name and age, date of surgery, and site of specimen. In addition, Surgical Pathology personnel write the Surgical Pathology identification number on the requisition form, and the repository biologist measures the tumor before it is divided, indicating the size of the tumor and the repository specimen-specific identification numbers on the requisition form. The BTSR biologist makes a copy of this form in the Pathology Laboratory and takes it to the BTSR along with the specimens. These data will eventually be entered into the BTSR database.

The following tests are routinely carried out on all breast biopsy samples at KUMC:

(1) estrogen and progesterone receptor analysis by ELISA method, carried out by Roche Laboratories, using at least 0.5cc of fresh tumor;

(2) estrogen and progesterone receptor analysis by immunocytochemistry read on image analyzer CAS-200, using frozen sections cut from the frozen-section tissue from which the initial diagnosis was made;

(3) proliferation antigen of Ki-67 using a frozen section cut from the frozen-section tissue from which the initial diagnosis was made;

(4) ploidy analysis by flow cytometry, using at least 0.5cc of fresh tumor;

(5) ploidy analysis by image analyzer CAS-200, using cells scraped from the tumor; and

(6) actual Surgical Pathology analysis, including a thorough analysis of tumor characteristics, histological type, histological grade, size, etc.

BTSR personnel can retrieve the results of all these tests as soon as they are available and enter the information into the BTSR database, as described below in Cataloging and Storage. Results from test (1) above are obtained from the Clinical Laboratory and test (4) results from the Flow Cytometry Laboratory, while those of the remaining tests are obtained from the Surgical Pathology Department.

SERUM SAMPLES

Blood samples both from women having breast surgery and from women at the KUCC High Risk Breast Clinic will be submitted to the BTSR. The procedure described below is followed for each group of women.

Three days before a patient is scheduled to have breast surgery, she is required to go to the Outpatient Laboratory to have her blood drawn for various presurgical tests. It is the BTSR biologist's responsibility to secure the schedule of these visits in advance from the surgeons' scheduling nurse and to advise the Outpatient Laboratory to draw one extra vial of blood from each of these patients for the BTSR. The BTSR biologist is stationed in the Outpatient Laboratory at the time of each of these appointments to be sure that this extra blood is drawn and to label the blood vials with the proper outpatient laboratory labels, which include the patient's name and hospital patient identification number.

In addition, the BTSR biologist gives the patient consent forms for donating blood to the BTSR, asking the patient to sign these and to complete the Personal Health History questionnaire described in detail below under Storage and Cataloging. After the patient completes the questionnaire, the BTSR biologist writes the six-digit specimen-specific identification number on the upper right-hand corner of the front page of the questionnaire.

Women who are considered at high risk for breast cancer are eligible to participate in the KUCC High-Risk Breast Clinic. In general, eligible women include those between 30 and 55 years of age who have at least one of the following conditions: a first-degree relative who has had breast cancer, or, in herself, precancerous mastopathy or prior node-negative breast cancer in one breast.

The High-Risk Breast Clinic is located at the KU Cancer Center Comprehensive Outpatient Diagnostic and Treatment Center. During each patient's first visit to the clinic, blood is drawn for various medical tests. The BTSR biologist is responsible for securing the schedule of these visits in advance and

advising the clinic to draw one extra vial of blood from each new patient for the Serum Repository. The identical procedure described above for securing the blood and completed questionnaire from breast surgery patients at the Outpatient Laboratory is also followed for new patients seen at the High-Risk Breast Clinic.

When blood specimens are received at the BTSR, the biologist processes the blood before the specimens are cataloged and stored in the freezer. After spinning down the reamed whole clotted blood in a refrigerated centrifuge, she removes the vial cap and, with a sterile pipette, divides the sera into 1.5-ml aliquots in the polypropylene containers. Each container is then labeled with the proper bar-code label and snap-frozen. Three times a day, the labels are scanned and the appropriate data entered into the Biopsy Serum Table, the Reduction Mammoplasty Serum Table or the High Risk Serum Table, depending on the source of the serum.

The specimen-specific number on the bar-code label will have been assigned to all specimens obtained. The six-digit identification number is identical to the number assigned to the tissue specimen for the same patient, when applicable.

LYMPHOCYTE SAMPLES

The BTSR has the capacity to separate and freeze lymphocytes from peripheral blood when a special request is received. Blood will be collected in heparin- or EDTA-containing tubes. A 10-ml tube is necessary. Preferably, two hours after blood collection, the procedure shown on p. 9 should be followed.

After all serum and lymphocytes are separated and labeled, the BTSR biologist then stores the tissue and serum samples in the freezer and records all data regarding storage location in the Location Table of the database. These data include specimen identification number and sample location, including freezer shelf, rack, box and cubicle number. This will allow the BTSR staff to locate all specimens quickly and easily.

STORAGE AND CATALOGING

When a tissue sample is received at the KUCC-BTSR, specimen bar codes are scanned into the Biopsy Table, the Healthy Adjacent Table, or the Reduction Mammoplasty Table of the Repository Database, as appropriate; the unique hospital patient identification number, the date that the specimen is received by the BTSR, the hospital of origin, the total amount of tissue, the surgical date, and all other data shown on the surgical requisition form that accompanies each specimen are then keyed in.

All specimen-specific and patient-specific data are maintained in the computerized Repository Database Management system, developed by the Program Database Leader using FoxPro for Windows, a database management software package. FoxPro is a relational database system that allows for various files in the system to be linked by means of key fields. In the Repository Database, the key fields are the unique specimen number and a combination of the hospital patient identification number and the hospital number. This combination serves as a unique patient identifier. Any or all of the tables within the database are linked using these three fields.

When a patient questionnaire is delivered to the Repository, it is initially labeled with the bar code showing the six-digit identification number for specimens from that patient. The questionnaire labels are then scanned and the data entered into the Demographic/Life Style Table. Responses to this questionnaire will be extremely valuable to many research investigators who will be using the BTSR breast specimens. The data requested include demographic, physical, and lifestyle information. Specifically, questions concern age, racial/ethnic background, marital status, religion, weight, height, education, occupation, family income, family history of breast cancer, age at first period, and menopausal, childbirth, lactation and alcohol history. To maintain confidentiality, all questionnaires are filed and locked up in a secure location after the data are entered into the database.

Results

To date, 9 malignant breast tumors have been cataloged and stored in the KUCC-BTSR, many of them with multiple samples for investigator use. Additionally, 4 nonmalignant specimens (e.g., fibroadenoma, fibrocystic, etc.) have also been collected. At present, only one normal breast tissue specimen (from breast reduction) has been obtained. These data are summarized (pg. 11,12).

Of the 27 blood serum samples collected so far, 19 were obtained from patients with a breast malignancy removed, and 8 from patients who had nonmalignant breast tissue. Lymphocytes were sampled until July 1995 according to isolation procedures shown on (p. 9). Of the 15 lymphocyte specimens collected, 9 were obtained from patients with a breast cancer removed, and 6 from patients who had nonmalignant breast tissue. These data are summarized (pg. 11,12).

It is pertinent to note that the original pathology protocol for obtaining breast cancer specimens has been changed; that is, reducing the Pathology requirement from utilization of 2.0 cm of breast tumor sample to 1.5 cm (p. 10). This allows KUCC-BTSR to collect greater numbers of breast cancer specimens than previously, since breast cancer specimens were frequently only 2.0 cm in size and thus unavailable for KUCC-BTSR collection.

Conclusions

In the next year, the following goals are to be fulfilled by KUCC-BTSR.

1. Increase collection of normal breast tissue from breast reduction surgery.

2. Call for breast, serum, and lymphocyte proposals from investigators at KUMC (Kansas City), Kansas State University (Basic Cancer Center), and KUMC (Wichita) (Women's Health Institute). A multidisciplinary review committee for this purpose has been assembled (p. 8) now that the KUCC-BTSR is completely functional.

3. Expand collection of human tumor specimens to ovarian and endometrial cancers and corresponding normal tissues. Since a number of KUMC investigators have research interests in cancers at these organ

sites, it seems useful to expand cancer research studies at KUMC by making these tumors available to all interested investigators.

Appendix

KUCC-BTSR COMMITTEE ON HUMAN TISSUE SPECIMEN USAGE

William Jewell, M.D. - Surgeon (breast), Professor
Jonathan J. Li, Ph.D. - Director, BTSR Core Facility, Professor
Sara Antonia Li, Ph.D. - Hormonal Carcinogenesis Researcher, Associate Professor
Janet Woodruff, M.D. - Pathologist, Assistant Professor
Walter Imagawa, Ph.D. - Breast Cancer Researcher and Associate Director, BTSR Core Facility, Assistant Professor
Carol Fabian, M.D. - Medical Oncologist (breast), Professor
Cooley Pantazis, M.D. - Chief, Surgical Pathology, Professor

Lymphocyte Separation

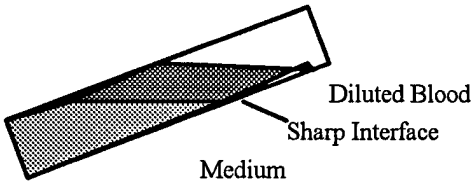
Collect blood in a 10ml heparin or EDTA containing vacutainer tubes.
Preferably, two hours after blood collection, follow this procedure:

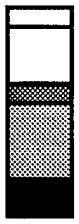
1. Pipet 4-5mL Histopaque-1077 into each of four 15mL centrifuge tubes;
2. Draw 3mL Hank's Solution into pipet, then 2-3mL whole blood and place in 15mL centrifuge tube. Add an additional 4mL Hank's Solution to tube, cap and mix by gently inverting tube. Prepare 4 tubes this way;
3. Tilt tube in #1 and add blood mixture so as to create a sharp interface;
4. Centrifuge at 400 x g (approx. 1400 rpm) for 30 minutes at room temperature;
5. After the centrifugation, draw off the opaque interface, being careful not to collect any of the medium below, and transfer to 15mL centrifuge tube containing approximately 5mL Hank's Solution. Mix by gently inverting capped tube, then fill tube with Hank's Solution;
6. Centrifuge at 250 x g (approx. 1000 rpm) for 10 minutes at room temperature;
7. Discard supernatant;
8. Resuspend pellet in 5 mL of Hank's Solution (mix using pipet - aspirations and vortexing);
9. Centrifuge at 250 x g (approx. 1000 rpm) for 10 minutes at room temperature;
10. Discard supernatant;
11. Resuspend pellet in 0.5 mL of Hank's Solution
12. Determine cell count using Crystal Violet (Stain 0.05mL cell solution with 0.45mL Crystal Violet and vortex for 20 sec.); Count number of stained cells in hemocytometer (determine total cells);
13. Put cells in bar code-labelled vial;
14. Freeze at -80°C.

Notes: Steps 5-11 are washing steps only.
For questions contact Dean Merkel in Hematology (pager #7014).
Blood dilution in step #1 useful in preventing loss of white cells in high concentration of red cells.

Materials needed:

15 ml capped centrifuge tubes
Histopaque-1077
Hank's Solution
Crystal Violet
5mL pipets
1mL pipets
200uL micropipet





Revised protocol for Breast Biopsies with infiltrating carcinoma

1) Examine the tissue submitted for biopsy by carefully making serial cuts.You are looking for a lesion for frozen section diagnosis. Always contact the fellow or staff. Submit a small but diagnostic piece of tissue for frozen section diagnosis.

2) **Measure** the lesion, immediately make two parallel cuts from the lesion: one for frozen section and one put in formalin for permanent section. Do this before you start your frozen. The frozen should not take more than 5 minutes.

3) If frozen section diagnosis. ASK FOR THE QUADRANT WHERE THE TUMOR IS LOCATED:

- *Positive for invasive mammary carcinoma*, you will have to take into account the size of the tumor to know what to do.

A) If tumor measures <1 cm in-greatest diameter:

i)Scrape the tumor with, a surgical blade for i) image and ii) flow cytometry analysis. Put the scraped material in the middle of a superfrost slide (++ at one end). Using another superfrost slide, press gently on the material and pull the slide along the short axis. Let air dry, then fix in formalin for 30 min. and take the slides to image room together with a copy of the requisition.

ii)Scrape the tumor with, a surgical blade. Use a small amount of pressure when scraping. Usually 6 scrapes 3/tumor slice will give about 2 million cells. With each scrape immerse the scalpel blade in RPMI medium (pink-red liquid) and shake the blade until cells are dislodged. Repeat this procedure for each scrape.

B) If tumor measures between 1 and 2 cm in greatest diameter:

i, ii Same as above

iii) Put a 0.5 x 0.1 cm piece of tumor into RPMI medium. This will be used for ploidy by flow cytometry. Call ext. 3876 (Bill Justice).

iv) Put a 0.5 cm piece of tumor into labeled plastic bag (patient name, hosp.#, and Surg Path#) Freeze immediately in liquid nitrogen. This plastic bag goes to metabolic lab for ER/PR ELISA method

C) If tumor measures between > 2 cm in greatest diameter:

i,ii Same as above.

iii) Cut two 0.5 cm pieces of tumor put one into labeled plastic bags (patient name, hosp.#, and Surg Path#) Freeze immediately in liquid nitrogen. This plastic bag goes to metabolic lab for ER/PR ELISA method. Call ext. 7020. Put the second piece of tumor into RPMI medium. This will be used for ploidy by flow cytometry. Call ext. 3876 (Bill Justice).

iv)Submit a 0.5 tumor section for the breast tumor bank.

-*Positive for in-situ carcinoma*. DO NOT GIVE ANY TISSUE AWAY. It is our responsibility to find the invasive component.

- NDA or benign diagnosis. Put the entire specimen in formalin.

The next day order from the block with most amount of tumor ER, PgR, MIB-1 and Also order from the same block an extra cut for ploidy.

Malignant and Non-Malignant Tissue

Bar Code	Date of Procedure	Age of Patient	# Vials	Sample Location
100000	5/12/95	72	2	1A1.1-2
100001	5/17/95	76	1	1A1.3
100009	6/5/95	46	1	1A1.4
100011	6/5/95	28	7	1A1.5-6, 1B1.1-5
100018	8/16/95	43	13	1A1.7-19 (mastectomy w/non-malignant tissue)
100020	8/16/95	52	1	1A1.20
100022	9/8/95	47	5	1A1.21-25 (mastectomy - only 1A1.25 contains malignant tissue
000003	9/8/95	71	9	1A1.26-34 (mastectomy w/non-malignant tissue)
000011	9/25/95	60	2	malignant tissue
	9/1/95	60	2	malignant tissue
	9/20/95	46	3	non-malignant tissue
	10/18/95	75	1	non-malignant tissue

Normal Tissue

Bar Code	Date of Procedure	Age of Patient	# Vials	Sample Location
100010	6/5/95	26	4	1B1.6-9

Blood Serum

Bar Code	Date of Procedure	Age of Patient	# Vials	Sample Location	Sample Class
100000	5/12/95	72	4	2A1.12-15	Breast Cancer
100001	5/17/95	76	3	2A1.25-27	Breast Cancer
100002	5/19/95	51	2	2A1.1-2	Fibrocystic
100003	5/22/95	59	3	2A1.3-5	Fibrocystic
100004	5/22/95	68	3	2A1.6-8	Breast Cancer
100005	5/22/95	54	3	2A1.9-11	Breast Cancer
100006	5/24/95	57	3	2A1.16-18	Breast Cancer
100009	6/5/95	46	3	2A1.22-24	Breast Cancer
100011	6/5/95	28	3	2A1.19-21	Breast Cancer
100012	7/10/95	78	3	2A1.28-30	Breast Cancer
100013	7/19/95	56	3	2A1.35-37	Breast Cancer
100014	7/12/95	60	3	2A1.32-34	Breast Cancer (7)
100015	7/19/95	43	2	2A1.38-39	Breast Cancer
100016	8/11/95	39	3	2A1.41-43	Fibrocystic
100017	8/30/95	68	3	2A1.52-54	Breast Cancer
100019	8/16/95	42	3	2A1.48-50	Breast Cancer
100020	8/16/95	52	1	2A1.51	Breast Cancer
000022	8/9/95	65	3	2A1.61-63	Fibrocystic
100022	9/8/95	47	4	2A1.55-58	Breast Cancer
000001	9/6/95	45	3	2A1.65-67	Fibrocystic
000002	9/8/95	51	2	2A1.70-71	Fibrocystic
000003	9/8/95	71	1	2A1.72	Breast Cancer
000005	9/13/95	46	3	2A1.75-77	Breast Cancer
000006	9/13/95	70	2	2A1.78-79	Breast Cancer
000010	9/22/95	52	2	2A1.80-81	Fibrocystic
000011	9/25/95	60	3	2A2.3-5	Breast Cancer
000012	10/2/95	64	3	2A2.7-9	Fibrocystic

Lymphocytes

Bar Code	Date of Procedure	Age of Patient	# Vials	Sample Location	Sample Class
100014	7/12/95	60	1	2A1.31	Breast Cancer
100016	8/11/95	39	1	2A1.44	Fibrocystic
100017	8/14/95	68	1	2A1.45	Breast Cancer
100019	8/16/95	42	1	2A1.47	Breast Cancer
100020	8/16/95	52	1	2A1.46	Breast Cancer
000022	8/9/95	65	1	2A1.60	Fibrocystic
100022	9/8/95	47	1	2A1.59	Breast Cancer
000001	9/6/95	45	1	2A1.64	Fibrocystic
000002	9/8/95	51	1	2A1.68	Fibrocystic
000003	9/8/95	71	1	2A1.69	Breast Cancer
000005	9/13/95	46	1	2A1.73	Breast Cancer
000006	9/13/95	70	1	2A1.74	Breast Cancer
000010	9/22/95	52	1	2A2.1	Fibrocystic
000011	9/25/95	60	1	2A2.2	Breast Cancer
000012	10/2/95	64	1	2A2.6	Fibrocystic

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